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Supplemental information

Aedes aegypti sialokinin facilitates mosquito blood feeding and modulates host immunity and vascular biology

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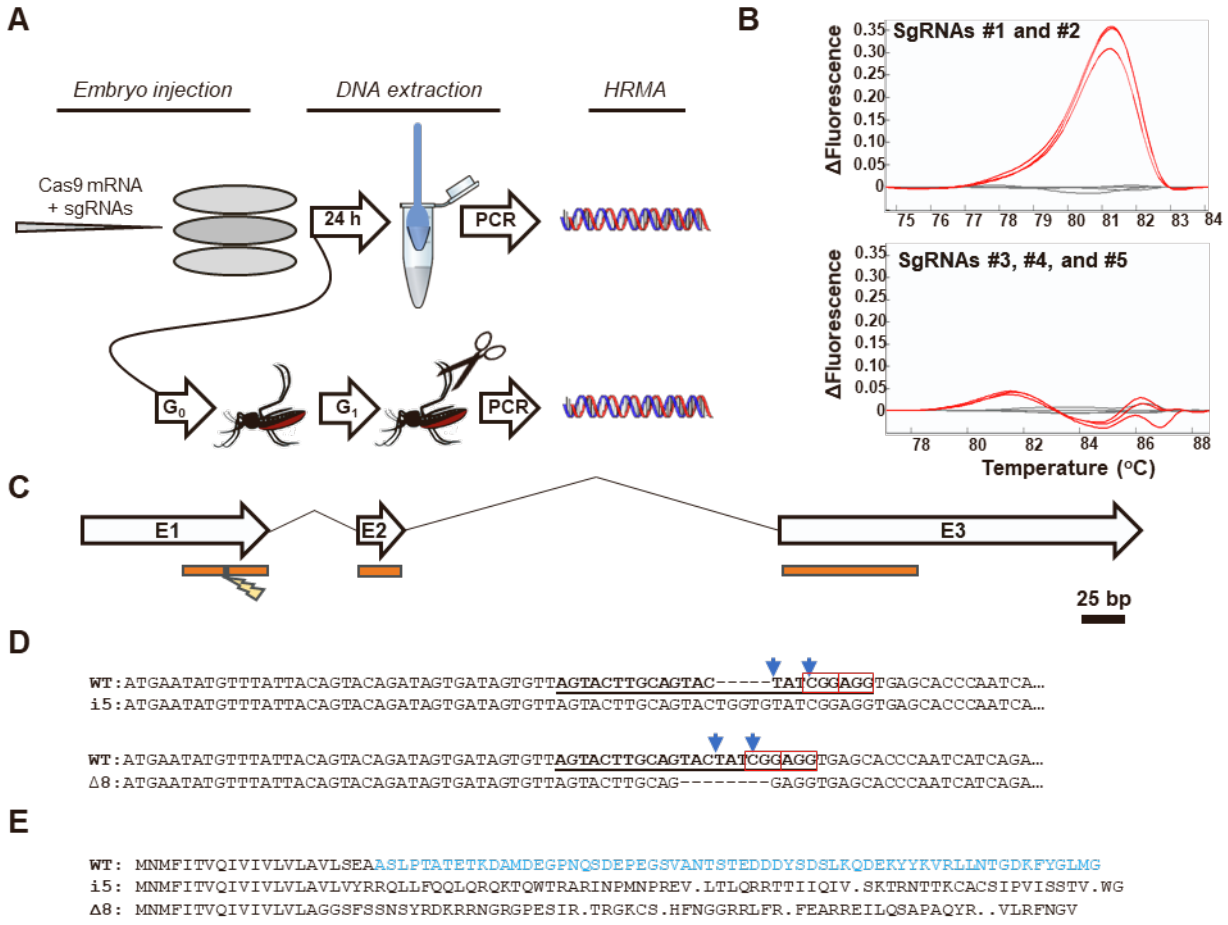
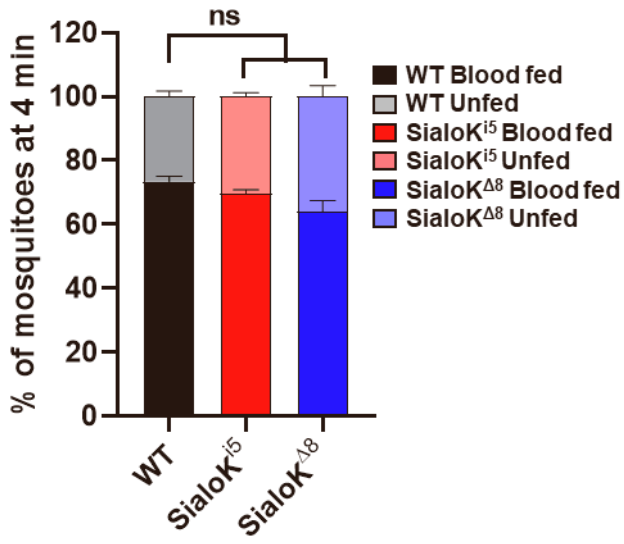


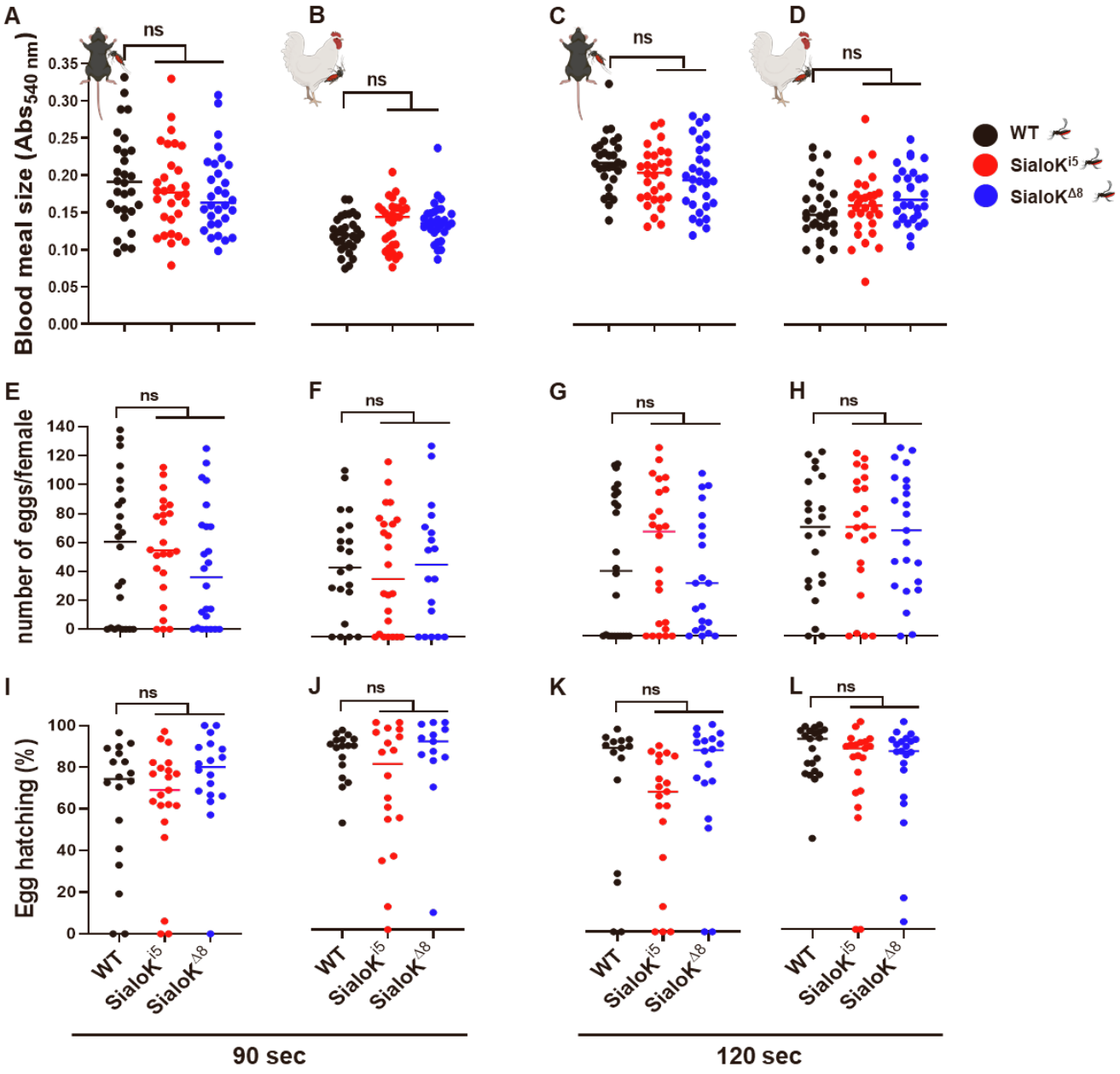
Supplementary Fig 1. CRISPR/Cas9 editing of *Aedes aegypti sialokinin*. Related to STAR methods.

(A) Experimental plan used to validate sgRNAs and identify loss-of-function mutations in *Ae. aegypti sialokinin* (AAEL000229). (**B**) High-resolution melt curve analysis (HRMA) of CRISPR/Cas9-treated (red) and WT (grey) embryos at 24 h post-injection. Three biological replicates were assayed per group. **(C)** *Sialokinin* gene model showing the approximate position of the ORF (orange bars) across each of three exons (E1, E2, and E3). Approximate scale is shown in base pairs (bp). Target sites of effective sgRNAs are indicated (lightning bolt). **(D)** Genomic DNA sequence of WT and CRISPR-induced mutations (i5 and Δ8). Underlined texts indicate the sgRNA target sequences and their genomic cleavage points (blue arrows). PAM sequences are indicated in red boxes. **(E)** Translation of the conceptual RNA from CRISPR-induced mutant alleles. Mature WT peptide without signal peptide is indicated in blue.



Supplementary Fig 2. Feeding rates of sialokinin-gene edited *Aedes aegypti* on mice for 4 min. Related to Figure 4.

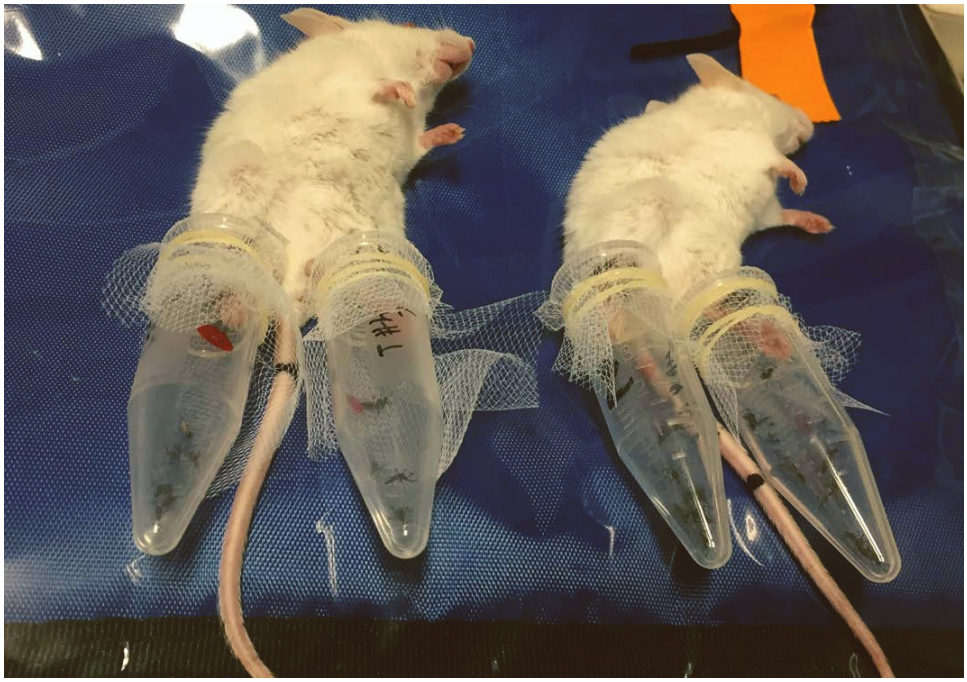
At longer times (4 min) the absence of sialokinin did not significantly reduce the feeding success of the KO mosquitoes (either sialoK^{i5} or sialoK$^{\Delta 8}$ lines) compared to WT group (WT). Feeding status was determined under a micro stereoscope and mosquitoes with any traces of blood in their midguts were considered as fed. The graph represents three independent experiments. Data are presented as mean ± SD of two independent experiments carried out by different operators. Each independent experiment consisted of two biological replicates of 100 mosquitoes each. Contingency analyses were performed by Fisher tests. ns: $P > 0.05$. GraphPad Prism v 7 (GraphPad Software) was used for statistical analysis.



Supplementary Fig 3. Blood meal intake and fecundity and fertility analysis of sialokinin-KO *Aedes aegypti.* Related to Figure 4.

Blood meal size of randomly selected fed mosquitoes (both partially and fully fed) in control group (WT) and gene edited mosquitoes (sialoK^{i5} and sialoK$^{\Delta 8}$). Graphs **A-D** show the amount of blood fed by individual mosquitoes after 90 and 120 sec of feeding on C57B/6L mice **(A, C)** or chicken **(B, D)**. Blood meal size of individual mosquitoes was measured by the total hemoglobin content in their midgut using Drabkin's reagent. 30 individual mosquitoes were assessed per group. Graphs **E-L** show the measurement of fecundity and fertility parameters after 90 or 120 sec of feeding in control group (WT) and KO mosquitoes (sialoK^{i5} and sialoK$^{\Delta 8}$). For fecundity and fertility, 24 independent mosquitoes were evaluated

per group. Fecundity **(E-H)** and fertility **(I-L)** parameters of sialokinin-KO *Ae. aegypti.* Fecundity was determined as the number of eggs laid per individual female fed on C57BL/6 mice **(E, G)** or chickens **(F, H)**. Fertility was determined as the number of larvae hatched per total number of eggs from mosquitoes fed on C57BL/6 mice **(I, K)** or chickens **(J, L)**. Results are indicated as the mean ± SEM. One-way ANOVA test was used to determine statistical significance using GraphPad Prism v 7 (GraphPad Software). For multiple comparisons, WT was selected as the control group; ns: $P > 0.05$.



Supplementary Fig 4. Mouse exposure to mosquito bites for cell recruitment study. Related to Figure 6.

Sedated animals were exposed to mosquito bites for cell recruitment studies. Animals were placed on a warm pad at 37 °C on supine position and their footpads were inserted in 5 mL-Eppendorf vials containing 10 starved mosquitoes.

Supplementary Table 3. sgRNA used for mosquito mutagenesis, Related to STAR methods.

sgRNA	**5'- T7-promoter - Gene-specific (20mer)-Scaffold-F (CRISPR) -3'**
sgRNA0229-1	GAAATTAATACGACTCACTATAGG**GTTAGTACTTGCAGTACTAT**GTTTTAGAGCTAGAAA
sgRNA0229-2	GAAATTAATACGACTCACTATAGG**AGTACTTGCAGTACTATCGG**GTTTTAGAGCTAGAAA
sgRNA0229-3	GAAATTAATACGACTCACTATAGG**GGGTCAGCTACACTTCCCTC**GTTTTAGAGCTAGAAA
sgRNA0229-4	GAAATTAATACGACTCACTATAGG**TAATCGTCGTCCTTCGTTGA**GTTTTAGAGCTAGAAA
sgRNA0229-5	GAAATTAATACGACTCACTATAGG**AAAGTGCGCCTGCTCAATAC**GTTTTAGAGCTAGAAA
Scaffold-R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGC TATTTCTAGCTCTAAAAC